EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
Screening Carriers Used in Disinfectant Efficacy Testing

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1.0 SCOPE AND APPLICATION:

1.1 This protocol describes the methods used to physically and biologically screen carriers for the AOAC Use-dilution Test Method, and physically screen carriers used in the AOAC Confirmatory Tuberculocidal Test Method, the AOAC Germicidal Spray Products Test Method and the Germicidal Towelette Product Test.

2.0 DEFINITIONS:

2.1 AOAC = AOAC International

2.2 OD = outside diameter

2.3 ID = inside diameter

2.4 DI water= De-ionized water

3.0 HEALTH AND SAFETY:

3.1 Personal protective equipment should be used to handle BTC 835.

3.2 All manipulations of the test organism are required to be performed in accordance with biosafety practices stipulated in SOP MB-01, Lab Biosafety.

4.0 CAUTIONS:

4.1 All carriers used for disinfectant testing must be screened in advance according to procedures outlined in this protocol.

4.2 Strict adherence to the protocol is necessary for the validity of the test results.

5.0 INTERFERENCES:

5.1 Examples of carriers passing and failing physical screens are studied during the training process. The physical screening task is assigned only to personnel who display consistent decision-making for passing and failing carriers.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 BTC 835: 50% n-Alkyl (50% C_{14}, 40% C_{12}, 10% C_{16}) Dimethyl Benzyl Ammonium Chloride

7.2 Stainless Steel Ring Carriers - polished cylinders, 8±1 mm OD, 6±1 mm ID, length 10 ±1mm; type 304 stainless steel, SS 18-8 (S & L Metal Products Corp., 58-29 57 Drive, Maspeth, NY, 11378).

7.3 Synthetic Hard water 200 ppm.

7.4 Sodium hydroxide (NaOH) 1N solution.

7.5 Phenolphthalein 1% (w/v) solution in ethanol.

7.6 Porcelain Carriers, 8±1 mm OD, 6±1 mm ID, 10±1 mm length (Fisher Catalog No. 07-907).

7.7 Glass Slide Carriers, 25 mm^2 microscope slides (Fisher Catalog No. 0002724)

8.0 INSTRUMENT OR METHOD CALIBRATION: Not applicable

9.0 SAMPLE HANDLING AND STORAGE:

9.1 BTC 835 should be stored at room temperature in a cabinet designed to contain flammable materials.

9.2 BTC 835 is diluted. Dilution should be performed within three hours of testing and diluted product should be kept stored at room temperature.

10.0 PROCEDURE AND ANALYSIS:

10.1 For the stainless steel carriers used in the AOAC Use-dilution Test Method, proceed as follows:

10.1.1 Physical Screening: Carriers are examined individually for

scratches, nicks, spurs, and discolorations. Carriers that do not pass the screening process are not used in testing and are returned to the manufacturer for repolishing. Record screening results in the Physical Screening of Carriers Record form (see 16.1).

10.1.2 Cleaning: Soak the ring carriers overnight (approx. 12 hr) in 1N NaOH and rinse several (3-4) times with tap water. Collect a portion of the last rinse water and add 2-3 drops of phenolphthalein 1%. If any NaOH remains, the phenolphthalein turns pink, indicating the need for additional rinsing. Continue to rinse the carriers until the addition of phenolphthalein does not produce a color change. Rinse twice more with DI water.

10.1.3 Distribute the carriers into 25 mm x 150 mm test tubes, 24 per tube.

10.1.4 Cover the carriers with about 20 ml of 0.1% asparagine solution and cap with Morton closures.

10.1.5 Fill out a media/reagent preparation sheet and assign a preparation number (see SOP QC-15, Media Prep and Sterilization Run Numbers). Autoclave at 121°C for 20 min; cool and store at room temperature for 3 months.

10.1.6 Biological Screening: Before the stainless steel carriers can be used for use-dilution testing, each individual carrier must be screened biologically. This is accomplished by performing an AOAC Use-Dilution test (see SOP MB-05, Use Dilution Method) on each carrier using a 48-54 hour old culture of *Staphylococcus aureus*.

10.1.6.1 BTC 835 is diluted to 500 ppm with synthetic hard water (200 ppm) and used as the test disinfectant.

10.1.6.2. All assays occur at 20±1°C, without organic soil, and with a ten minute exposure period to BTC-835.

10.1.6.3 Positives (with growth) are confirmed by Gram stain. If Gram-positive cocci are observed, the carrier fails and will not be used for testing.

10.1.6.4 If an organism other than Gram positive cocci is identified, the carrier is cleaned following the procedure in 10.1.2, and biological screening is performed again. If a carrier shows a negative result (no growth), it is recleaned as noted in 10.1, and used for product testing.

10.1.6.5 Fill out a media/reagent preparation sheet and assign a preparation number to the "pass" carriers (see SOP QC-15, Media Prep and Sterilization Run Numbers).

10.1.6.6 Record screening results in the Use-dilution Test Results Sheet for Screening Carriers (see 16.3).

10.2 For the porcelain carriers used in the AOAC Confirmatory Tuberculocidal Test Method (see SOP MB-07, Confirmatory Tuberculocidal Method), proceed as follows:

10.2.1 Physical Screening: Porcelain carriers are examined individually for scratches, nicks, spurs, and discolorations. Carriers that do not pass the screening process are not used in testing. Record screening results in the Physical Screening of Carriers Record Form (see 16.1).

10.2.2 Cleaning: Soak the porcelain carriers overnight (approx. 12 hr) in 1N NaOH and rinse several (3-4) times with tap water. Collect a portion of the last rinse water and add 2-3 drops of phenolphthalein 1%. If any NaOH remains, the phenolphthalein turns pink, indicating the need for additional rinsing. Continue to rinse the carriers until the addition of phenolphthalein does not produce a color change. Rinse twice more with DI water.

10.2.3 Place the cleaned porcelain carriers in 20 mm X 150 mm test tubes, 12 per tube.

10.2.4 Fill out a media/reagent preparation sheet to assign a preparation number to the carriers (see SOP QC-15, Media Prep and Sterilization Run Numbers) and autoclave at 121°C for 20 minutes; cool; store at room temperature for up to 6 months.

10.2.5 All porcelain carriers used in product testing are discarded.

10.3 For the glass slide carriers used in the AOAC Germicidal Spray Products Test Method (see SOP MB-06, Testing Spray Disinfectants) and Germicidal Towelette Product Test (see SOP MB-09, Testing Towelette Disinfectants), proceed as follows:

10.3.1 Physical Screening and Cleaning: Visually screen glass slide carriers for scratches, chips or cracks and discard those which are damaged or defective.

10.3.2 Prior to carrier preparation for testing, rinse the carriers once with DI water, rinse three times with 95% ethyl alcohol, and finally rinse three times with DI water.

10.3.3 Drain and allow carriers to dry before use. Record screening results in the Physical Screening of Carriers Record form (see 16.1).

10.3.4 Place one glass slide carrier into a petri dish with 2 pieces of Whatman No. 2 filter paper. Fill out a media/reagent preparation sheet to assign a preparation number to a set of carriers (see SOP QC-15, Media Prep and Sterilization Run Numbers).

10.3.5 Autoclave for 45 minutes at 121°C with a 30 minute dry cycle; cool; store at room temperature.

10.3.6 All glass slide carriers used in testing are discarded.

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the appropriate screening record forms (see 16.0). Completed forms are archived in notebooks kept in locked file cabinets in D217. Only authorized personnel have access to the locked files. Archived data is subject to OPP's official retention schedule as outlined in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The OPP Microbiology Laboratory conforms to 40 CFR Part 160, Good Laboratory Practices. Appropriate quality control measures are integrated into each SOP.

13.2 For quality control purposes, the required information is documented on the appropriate forms (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Stainless steel and porcelain carriers that fail physical screening will not be used in testing.

14.2 Stainless steel carriers which fail the biological screening will not be used for testing. If an organism other than a Gram positive coccus is identified as a contaminant resulting in a "positive" carrier, then the carrier may be cleaned and rescreened.

14.3 All stainless steel carriers used for product testing that subsequently give a positive (with growth) result must be cleaned and biologically rescreened in the same manner before reusing. In addition a new preparation number will be assigned to these carriers.

15.0 REFERENCES: None

16.0 FORMS AND DATA SHEETS:

16.1 Physical Screening of Carriers Record

16.2 AOAC Use-Dilution Test Information Sheet For Biological Screening of Carriers

16.3 AOAC Use-Dilution Test Results Sheet For Carrier Screening

Physical Screening of Carriers Record

OPP Microbiology Laboratory

Carrier Information			Screening Results			
Type	Vendor	Control Number	Date Screened/Initials	No. Pass	No. Fail	Prep # Assigned

AOAC Use-Dilution Test Information Sheet For Biological Screening of Carriers
OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.	1839-32	SOP	
Name	BTC-835	Test Date	
Sample No.		Comments/Modifications:	
Lot No.			

TEST PARAMETERS/Confirmed by:_____			
Use Dilution	Specified	As Prepared/ Date/Initials	
		/ /	
Neutralizer	Specified		
Temperature	Specified	Chiller Unit Display	Test tube Water Bath
		Before: After:	Before: After:
Contact Time	Specified	As Tested	
Other Parameters	Specified		

TEST MICROBE INFORMATION/Confirmed by:_____				
Test Microbe	*Staphylococcus aureus*	48-54 Hour Culture		
Org. Control No.		Date/Time	Initiated	Harvested
Avg. CFU/Carrier				

REAGENT/MEDIA INFORMATION/Confirmed by:_____			
Reagent/Media	Prep. No.	Reagent/Media	Prep. No.

AOAC Use-Dilution Test Results Sheet For Carrier Screening

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Organism	

CARRIER INFORMATION (to be completed by Analyst)		
Carrier Drop Time Interval	Carrier Set	Analyst

TEST RESULTS									
Date Recorded/Initials									
Primary Subculture/Secondary Subculture (contains carrier)									
1	2	3	4	5	6	7	8	9	10
/	/	/	/	/	/	/	/	/	/
11	12	13	14	15	16	17	18	19	20
/	/	/	/	/	/	/	/	/	/
21	22	23	24	25	26	27	28	29	30
/	/	/	/	/	/	/	/	/	/
31	32	33	34	35	36	37	38	39	40
/	/	/	/	/	/	/	/	/	/
41	42	43	44	45	46	47	48	49	50
/	/	/	/	/	/	/	/	/	/
51	52	53	54	55	56	57	58	59	60
/	/	/	/	/	/	/	/	/	/
Results Summary			Number of Carrier Sets with Growth						
			Number of Carrier Sets without Growth						
Modifications/Comments:									